Project 4 – Marine Mammal Color Vision and Fishing Tackle Avoidance (Kean University)

Project Goal and Objectives

The goals of this project are threefold:

1. Sequence the rod and cone visual pigment coding regions using genomic DNA from the most susceptible and threatened species, identify the spectral tuning amino acid substitutions, incorporate these substitutions into the visual pigment model, express the visual pigment and examine the resulting absorption spectra.
2. Obtain fresh tissue yielding quality mRNA from the most susceptible and threatened species (or closely related species) to develop a second visual pigment model.
3. Identify the wavelength(s) of light that will give each particular species the highest level of contrast to their visual perception.

Project 4 Final Report (J. Fasick)

Abstract

Fishing equipment, including lines and nets, have been involved with relatively high numbers of incidental captures and deaths of cetaceans. This suggests that these marine mammals may be unable to visually detect the presence of these underwater obstacles. This research focuses on marine mammal vision, specifically, determining the wavelengths of light (color) to which the eye is most sensitive. Once these wavelengths are determined, it would allow fishing tackle to be constructed or appended with a color that a particular species would be able to detect visually and possibly avoid. To approach this problem, genomic DNA from high incidental capture species will be used to identify the amino acids at key positions in the rod and cone opsin protein components of the visual pigments. These residues will then be incorporated into a spectral tuning model to determine the absorbance maximum of the resulting visual pigment. Alternatively, if fresh eyes are available, total RNA will be used as a template to express the visual pigments and identify the absorbance maxima. This research may be of particular value in easing the incidental captures and deaths of *Eubalana glacialis* (North Atlantic right whale) by fishing tackle used in the Western North Atlantic lobster fisheries.

Goals

The goals of this project were 3-fold:

1) Sequence the rod and cone visual pigment coding regions using genomic DNA from the most susceptible and threatened species, identify the spectral tuning amino acid substitutions, incorporate these substitutions into the visual pigment model, express the visual pigment and examine the resulting absorption spectra.
2) Obtain fresh tissue yielding quality mRNA from the most susceptible and threatened species (or closely related species) to develop a new visual pigment model specific for the mysticete whales.
3) Identify the wavelength(s) of light that will give each particular species the highest level of contrast to their visual perception.

Collaborations/Tissue & DNA Sources

To accomplish these goals, genomic DNA samples from 11 species of mysticete whales were acquired from NOAA NMFS, SWFSC. These samples are listed in Table 1 with the NMFS numbers listed.

We received an eye (NARW *E. glacialis* calf # CALO 0901) from William McLellan, University of North Carolina-Wilmington which has allowed us to directly clone and sequence the rod opsin coding region. We received a second eye from McClellan (NARW *E. glacialis* adult #EgNEFL1103) which was code 3 and may be used at a future date for anatomical analysis with Michael Moore at WHOI.

Dr. Thomas Cronin (Department of Biological Sciences, University of Maryland Baltimore County) and Dr. Mark Baumgartner (Biology Department, Woods Hole Oceanographic Institute) have assisted us in a project involving examining predator/prey relationships associated with vision with the right whale and its primary prey species, the calanoid copepod *Calanus finmarchicus*.

Dr. Benjamin Nickel (Department of Biochemistry, Brandeis University) is our most recent collaborator and has had great success in the expression and biochemical analysis of the right whale rod visual pigment.

Detailed Summary of Completed Work:

1) Estimated Absorbance Maxima of E. glacialis Rod and MWS Cone Visual Pigments

From DNA sequence alignments, we designed a set of degenerate and non-degenerate oligonucleotide primers for amplifying specific regions of the cetacean rod and long-wavelength sensitive (LWS) cone opsins. PCR reactions were first done on bottlenose dolphin genomic DNA to determine correct size and quality of the products. Subsequent PCR products from right whale gDNA (NOAA lab ID numbers 15112, 28311 and 13086) were cloned by ligation into a cloning vector and transformation into bacteria. Colonies containing inserts were picked, cultures grown overnight and PCR amplified again to confirm the presence of inserts. Clones positive for inserts were sequenced. The results from this work were successful with the sequencing of all three amino acid positions in the rod opsin (83, 292 and 299) and a single amino acid position in the LWS cone opsin (292). During this process we identified NOAA sample 15112 as being Tursiops in its origin with mislabeling of this sample most likely occurring at the NOAA labs. Regardless, results from the animal samples 28311 and 13086 provided us with amino acid identity at the important spectral tuning positions mentioned above. We have identified the following amino acid substitutions in the right whale rod visual pigment gene: N83, A292 and S299 with an estimated absorbance maximum of 499 nm. The right whale LWS cone visual pigment possesses the amino acid substitution S292 with a predicted absorption maximum of 524 nm. This work was recently published in the peer-reviewed journal *Marine Mammal Science* [MARINE MAMMAL SCIENCE, 27(4): E321–E331 (October 2011); see Appendix 5].

We had the good luck of receiving a fresh right whale eye (NARW *E. glacialis* calf # CALO 0901) from which we were successful in extracting quality total RNA. We have been able to clone the full length rod opsin, confirm its sequence against genomic DNA, and have successfully expressed this pigment in tissue culture. We have determined that the right whale rod visual pigment has a dark adapted absorbance maximum of 493 nm when compared to that from bovine (496 nm). When the difference spectra absorbance maxima are normalized to bovine rhodopsin reported absorbance maxima of 500 nm we see that the expressed right whale rod visual pigment has an absorbance maxima of 498 nm, nearly identical to the value predicted in our manuscript of 499 nm described above.

We have had limited success in PCR amplifying the middle-wavelength sensitive (green) cone opsin sequence from CALO 0901. To date we have sequenced the regions spanning exons 2-5 from a gene

that consists of 6 exons. Our analyses of three different PCR products show inversions, deletions and duplication events in exon 4 of the mRNA. These are very unusual mutations resulting from improper splicing events and would not allow for a functional pigment to be expressed. Without the expression of a functional MWS cone pigment, the photoreceptor cells atrophy and are lost. If this *is* the case, the animal would be a rod monochromate and possess little or no photopic (day-time) vision. Presently, we are not prepared to state that this species, nor this individual, is lacking a functional MWS cone class. Rather, we have not been able to identify a wildtype like MWS cone opsin sequence from this individual. We are currently examining the genomic DNA from this individual to compare it to other genomic samples that we have acquired through NMFS SWFSC. However, the quickest and most efficient way of answering this question would be to repeat our analysis of the retinal mRNA with another individual, preferably an adult.

2) Spectral Placement of the North Atlantic Right Whale (Eubalaena glacialis) Visual Pigments and Their Potential Role in Detecting Concentrations of the Calanoid Copepod Calanus finmarchicus.

To assess the role that vision may play in the ability of the right whale to detect its primary prey species, the calanoid copepod *Calanus finmarchicus*, we have directly determined the absorbance spectrum of the *E. glacialis* rod visual pigment as well as the transmission spectra of the *C. finmarchicus* carotenoid pigments. We determined that the *E. glacialis* rod visual pigment absorbs light maximally at 493 nm while a previous study positions the absorbance maximum of the *E. glacialis* cone visual pigment at 524 nm. Microspectrophotometric measurements of the *C. finmarchicus* carotenoid pigments result in transmission spectra with minima that match very well with the *E. glacialis* rod and cone visual pigment absorbance maxima, suggesting that these carotenoids would effectively block visible sidewelling or downwelling light. We conclude that the *E. glacialis* visual pigments are ideal for detecting concentrations of copepods in silhouette against natural lighting.

After opsin expression, reconstitution with 11-*cis* retinal and purification, the right whale rod visual pigment, rhodopsin, was shown to have a λ_{max} = 493 nm (Figure 1). In side-by-side purification experiments, the spectrum of right whale rhodopsin was shown to be slightly blue-shifted (3 nm) from that of the more commonly studied bovine rhodopsin (λ_{max} = 496 nm, see Figure 3) and confirmed the previous λ_{max} estimate of right whale rhodopsin. Full length right whale MWS cone opsin cDNA was not successfully PCR amplified from first strand cDNA samples. However, the right whale MWS visual pigment has previously been estimated to have a λ_{max} value of 524 nm and is plotted in Figure 1.

Microspectrophotometric scans of freshly mounted individuals of *C. finmarchicus* (Fig. 2) produced peak optical densities that commonly exceeded 2.0, even though regions selected for scanning were relatively clear compared to unscanned regions. All scans showed strongest absorbance in the wavelength region from 450 to 500 nm. When plotted as transmission spectra, as shown in Figure 1, transmission is greatest at wavelengths longer than 600 nm with transmission minima occurring between approximately 450 and 550 nm. The decreases in the transmission minima shown in Figure 1 are associated with increases in carotenoid pigment density, as the densest pigments produce the lowest transmission spectra (e.g., the maximum OD is 1.98 @ ~497 nm in the bottom curve; 1.56 @ ~ 478 nm in the middle curve; and 1.06 @ ~467 nm in the upper curve).

Our results show that the right whale rod and cone visual pigments are tuned to a region of the spectrum to detect underwater background light but would not be sensitive to wavelengths greater than 650 nm, the very region of the spectrum where the transmission maxima for the *C. finmarchicus* carotenoid pigments are positioned. In this situation, *C. finmarchicus* would produce a perfect high-contrast dark silhouette against the bright background space-light in either the horizontal or upward

visual axes. Previous investigations of the feeding behaviors of the right whale suggest that they are capable of detecting variations in prey density in both the horizontal and vertical directions, adjusting their foraging behavior to remain in areas of maximum copepod density. We can speculate that the spectral placement of the right whale visual pigments allow the whale to visually perceive prey concentrations with high spatial and temporal resolution, allowing for the effective adjustment of foraging behavior.

3) Expression and Direct Determination of E. glacialis Rod Visual Pigment Absorbance Spectrum

To better understand the spectral tuning properties of the cetacean rhodopsins, we analyzed expressed rhodopsins from the right whale, bottlenose dolphin, Sowerby's beaked whale and the domestic cow. Here we cloned, expressed, reconstituted expressed opsins with the chromophore 11-*cis* retinal, and purified the resulting visual pigments for analysis by spectrophotometry. The absorbance spectra of these visual pigments are seen in Figure 3 which clearly shows two groupings of pigments based on absorbance maxima. Both the cow and right whale rhodopsin spectra are grouped near each other with absorbance maximum (λ_{max}) values of 496 and 493 nm, respectively. Likewise, both the bottlenose dolphin and beaked whale rhodopsins are grouped near each other with λ_{max} values of 484 and 479 nm, respectively. The placement of these four spectra clearly demonstrates the differences between the right whale from the odontocetes in terms of the spectral sensitivity of the rod visual pigment, with the placement of the right whale rhodospin λ_{max} nearer to that of a terrestrial mammal than to that of the odontocetes. This is most likely due to adaptations resulting in the amino acid substitutions N83, A292, and S299 in the right whale rhodopsin that benefit foraging in a relatively shallow foraging photic environment. Thus, the right whale rhodopsin can be defined as being intermediate in its spectral sensitivity to the terrestrial and deep-sea rhodopsins.

4) Estimated Absorbance Maxima for Eleven Mysticete Whale Rhodopsins

As shown in Table 1 and Figure 4, we have identified the amino acid substitutions occurring in the rod opsin gene at amino acid positions 83, 292 and 299 for 11 extant baleen whales as well as those found in the sperm whale (*Physter macrocephalus*) used for comparison. Based on these amino acid substitutions, we were able to reconstruct the evolution of these substitutions (Figure 5) and estimate the absorbance maximum for each pigment as shown in Table 1. Estimating the absorbance maxima for these visual pigments was accomplished by sequence analysis of exons 1 and 4 of the rod opsin genes from 22 individuals representing 11 species from each of the four mysticete families (Fig. 4). DNA samples used for this analysis were supplied by NMFS SWFSC. Amino acid substitution, estimated absorbance maxima as well as the evolution of the opsin genes is described below. Interestingly, all but one of the mysticete rod visual pigment λ_{max} values described below can be described by the amino acid substitutions and resulting spectra shown in Figure 3.

4) Evolution of the Mysticete Whale Rhodopsins

As seen in Figure 5, the ancient cetacean rhodopsin included the amino acid substitutions N, S, A at positions 83, 292 and 299, respectively and had an absorbance maximum (λ_{max}) of 479 nm. All cetacean studied to date retain the amino acid substitution N83, except for the humpback whale which possesses D83. As the mysticete whales emerged, the Balaenidae acquired two amino acid substitutions (A292 and S299) resulting in a rhodopsin with λ_{max}=493 nm, a red-shifted value when compared to the odontocetes and most likely associated with foraging in relatively shallow waters. Interestingly, the pygmy right whale (*C. marginata*) retains the ancient amino acid substitutions found in odontocetes (λ_{max}=479 nm) associated with deep-sea foraging. Little is known of the foraging patterns of the pygmy

right whale, but it is not currently believed to be a deep-diving forager. As the Balaenopteridae and Eschrichtiidae emerged, the majority of the clades acquired the two amino acid substitutions S292 and S299 with λ_{max}=484 nm, a significantly blue-shifted rhodopsin when compared to Balaenidae, first identified and associated with delphinidae. Two exceptions are found with the gray whale (*E. robustus*) which incorporates the amino acid substitutions NAS (λ_{max}=493 nm) like the Balaenidae, and the humpback whale (*M. novaeangliae*) which incorporates the novel amino acid substitutions DSS (λ_{max}=492 nm). We found it very interesting that the Balaenopteridae and Eschrichtiidae rhodopsins were significantly blue-shifted. λ_{max} values of 484 nm have previously only been identified in the delphinidae, animals that dive routinely to several hundred meters to forage. It is not clear why these baleen whales would retain such a blue-shifted visual pigment other than the fact that the placement of the pigment serves well in the photic environment where they forage. The gray whale is a coastal species and the red-shift associated with its rhodopsin, when compared to the Balaenopteridae, makes sense considering the relatively shallow, coastal photic environment in which they forage. Likewise with the humpback whale foraging in relatively shallow photic environments when compared to the delphinidae.

5) Identification of wavelength(s) of light that will provide the right whale with the highest level of contrast in their visual perception.

All cetaceans lack a functional short-wavelength sensitive cone photoreceptor class relying solely on a single MWS cone photoreceptor for day-time photopic vision. Utilizing only a single cone photoreceptor class leaves these animals color blind in bright light conditions. Likewise, the single rod photoreceptor class does not provide color information under dim-light scotopic conditions. Generally speaking, the underwater photic environment to a cetacean does not appear blue, green or red as it may to the human eye. Rather, the cetaceans have adapted to this underwater photic environment with associated blue-shifts in the spectral sensitivities of both their rod and cone visual pigments. Depending on the depth at which individual species forage, the rod visual pigments may be only slightly blue-shifted in its spectral sensitivity as seen in the right, gray and humpback whales (λ_{max} values ≈495 nm) or extremely blue-shifted in their sensitivity as seen in the Balaenopteridae, Eschrichtiidae and Delphinidae (λ_{max} values ≈484 nm) and the Neobaelinidae, sperm and beaked whales (λ_{max} values ≈479 nm).

What does this blue-shift in spectral sensitivity mean in terms of vision and foraging? As the wavelength of maximum sensitivity decreases in cetacean rhodopsins, there is a strong correlation with an increase in depth of foraging. In essence, the deeper an individual species dives to forage, the more blue-shifted the rod visual pigment spectral sensitivity has become. This adaptation has been influenced by the filtering properties of oceanic waters with the removal of long-wavelength light with increasing depth. At several hundred meters depth, the available solar light is very narrow in terms of the visible spectrum and can be place around 480 nm, very near to the spectral sensitivities of the pelagic rods of the odontocetes as well as some mysticetes. At these depths the water color and all objects in the water column that reflect and transmit solar light appear blue to the human eye due to our ability to discriminate spectral hues utilizing our trichromatic cone sensitivities. To the cetacean eye, this same photic environment would appear bright due to the absorbance of the background light by the rods, but would be lacking in what we would describe as color. To the cetacean, objects within this photic environment that reflect/transmit the narrow blue wavelength band at depth would essentially be indiscernible from the background light. However, objects that absorb this blue background light would appear to the cetacean eye as a dark object against a bright background.

With this in mind objects that absorb light in the blue and green region of the spectrum and reflect or transmit light in the yellow, orange or red regions of the spectrum would provide the cetacean eye with the greatest amount of contrast to the background light. This is quite evident with the data that we provide in this report on the prey species *C. finmarchicus* and its red carotenoid pigments. If one were forced to pick a single spectral hue that would provide cetaceans with the greatest contrast underwater that color would be "red" (λ_{max}>700 nm). But essentially any wavelength longer than the wavelengths associated with each animals rod and cone spectra would offer good contrast depending on light conditions (photopic vs. scotopic). These values would be wavelengths longer than 625 nm under scotopic conditions and wavelengths longer than 650 nm under photopic conditions for most cetaceans. The human eye would describe wavelengths of 625 nm as "orange" and 650 nm as "orange" or "red", with λ_{max}>700 nm being described as "red".

Work has been initiated by the New England Aquarium to test these colors in the ocean to determine if right whales are able to detect and/or avoid them (see Scott Kraus, Project 5). Interestingly, when colored objects were videotaped at a depth of several meters, the object painted "red" provided the greatest contrast at distance more so than objects of different colors including white and black.

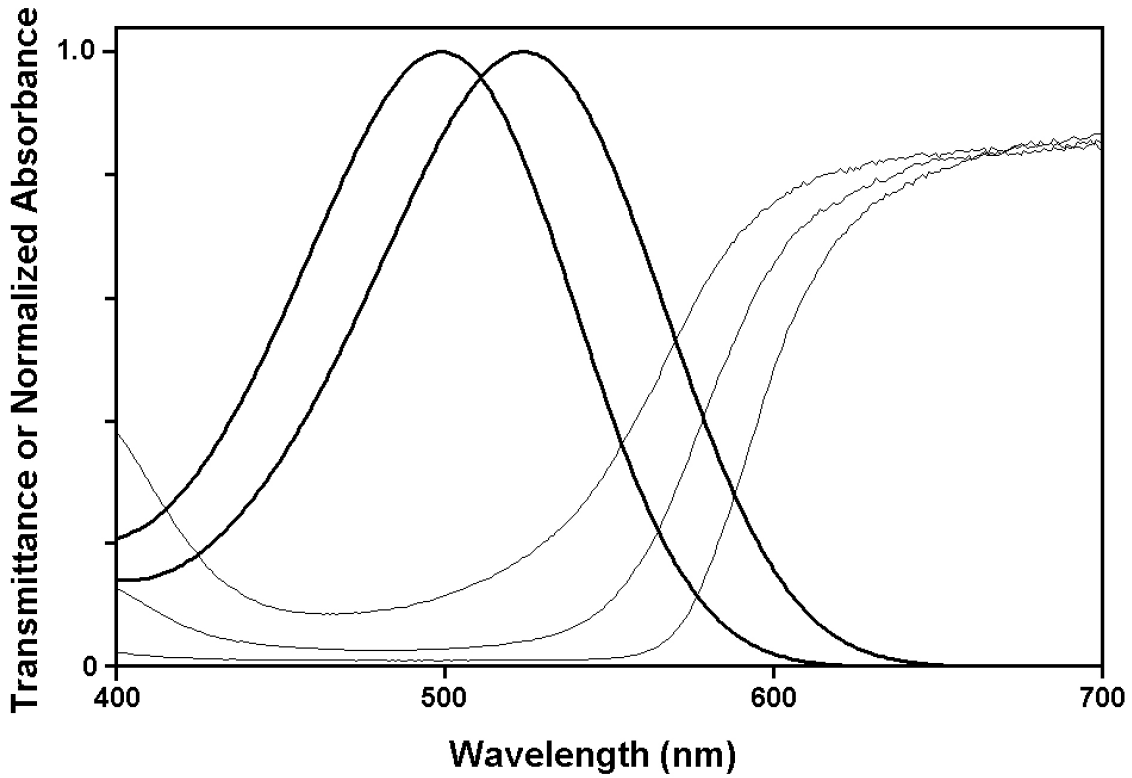

Figure 4-1. Normalized absorbance spectra of the rod and middle-wavelength sensitive (MWS) cone visual pigments of *Eubalaena glacialis* and transmission spectra of carotenoid pigments from *Calanus finmarchicus*. The absorbance spectra for the rod (λ_{max}=493 nm) and MWS cone (λ_{max}=524 nm) visual pigments are shown as dark traces. Normalized transmission spectra of typical carotenoid pigments from *C. finmarchicus* are shown as light traces.

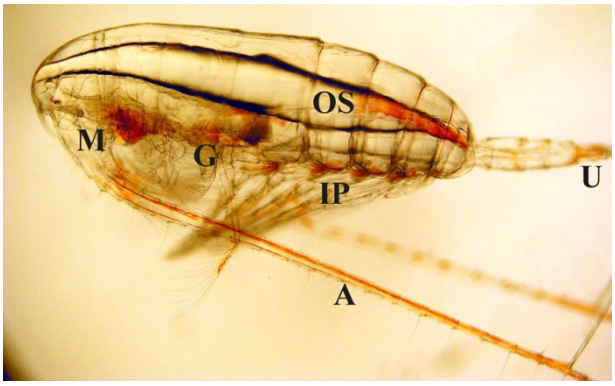

Figure 4-2. Calanoid copepod *Calanus finmarchicus*. Carotenoid pigments are associated with the posterior tip of the oil sac (OS), antennae (A), urosome (U), mouth (M), gut (G) and insertion points of the legs (IP).

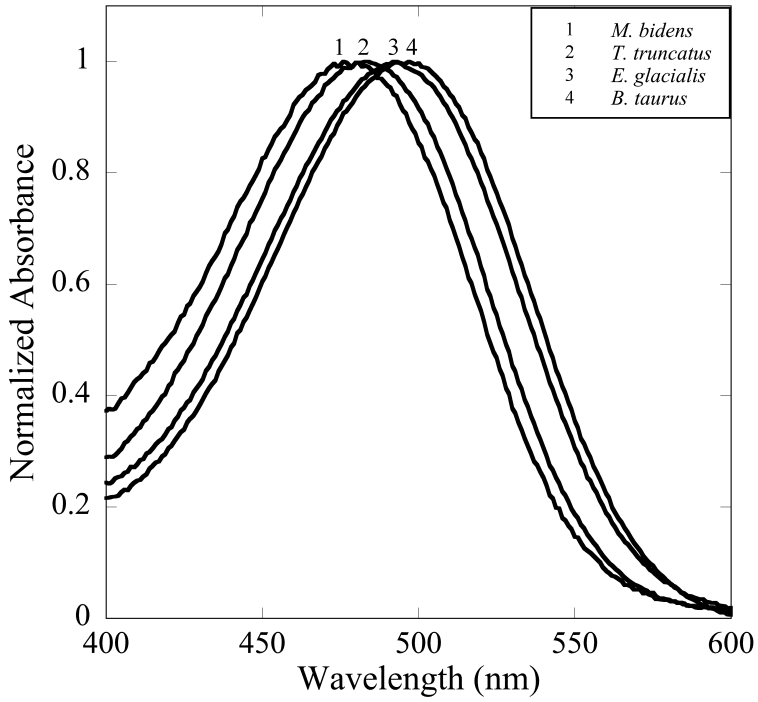

Figure 4-3. Absorbance spectra of dark adapted cetacean rhodopsins. Absorbance maxima are as follows: 1, *Mesoplodon bidens* 479 nm; 2, *Tursiops truncatus* 484 nm, 3, *Eubalaena glacialis* 493 nm; 4, *Bos taurus* 496 nm.

Table 4-1. Estimated Mysticete Rhodopsin Absorbance Maxima and Associated Amino Acid Substitutions.

Mysticete Rho Positions					
Animal	**Number**	**lmax**	**83**	**292**	**299**
Balaenidae					
B. mysticetus	44685	493	N (asparagine)	A (alanine)	S (serine)
B. mysticetus	50787	493	N (asparagine)	A (alanine)	S (serine)
E. australis	18928	493	N (asparagine)	A (alanine)	S (serine)
E. glacialis	CALO 0901	493	N (asparagine)	A (alanine)	S (serine)
Neobalaenidae					
C. marginata	5988	479	N (asparagine)	S (serine)	A (alanine)
C. marginata	5989	479	N (asparagine)	S (serine)	A (alanine)
Balaenopteridae					
B. musculus	43575	493	N (asparagine)	S (serine)	S (serine)
B. musculus	43758	484	N (asparagine)	S (serine)	S (serine)
B. physalus	43617	484	N (asparagine)	S (serine)	S (serine)
B. physalus	43963	484	N (asparagine)	S (serine)	S (serine)
B. borealis	30493	484	N (asparagine)	S (serine)	S (serine)
B. borealis	25386	484	N (asparagine)	S (serine)	S (serine)
B. edeni	30430	484	N (asparagine)	S (serine)	S (serine)
B. edeni	15911	484	N (asparagine)	S (serine)	S (serine)
B. edeni	30451	484	N (asparagine)	S (serine)	S (serine)
B. acutorostrata	23182	484	N (asparagine)	S (serine)	S (serine)
B. acutorostrata	5318	484	N (asparagine)	S (serine)	S (serine)
M. novaeangliae	11201	492	D (aspartic acid)	S (serine)	S (serine)
Eschritidae					
E. robustus	52434	493	N (asparagine)	A (alanine)	S (serine)
E. robustus	52435	493	N (asparagine)	A (alanine)	S (serine)
Delphinidae					
P. electra	cDNA	484	N (asparagine)	S (serine)	S (serine)
Physeteridae					
P. macrocephalus	cDNA	479	N (asparagine)	S (serine)	A (alanine)

Exon 1

	83
Eubalaena glacialis	GFPINFLTLYVTVQHKKLRTPLNYILLNLAVA**N**LFMVFGGFTTTLYTSLHA
Tursiops truncatus	GFPINFLTLYVTVQHKKLRTPLNYILLNLAVA**N**LFMVFGGFTTTLYTSLHA
Mesoplodon bidens	GFPINFLTLYVTVQHKKLRTPLNYILLNLAVA**N**LFMVLGGFTTTLYTSMHA
Bos taurus	GFPINFLTLYVTVQHKKLRTPLNYILLNLAVA**D**LFMVFGGFTTTLYTSLHG
Physeter macrocephalus	GFPINFLTLYVTVQHKKLRTPLNYILLNLAVA**N**LFMVFGGFTTTLYTSLHA
Balaenoptera borealis	SFPINFLTLYVTVQHKKLRTPLNYILLNLAVA**N**LFMVFGGFTTTLYTSLHA
Balaena mysticetus	GFPINFLTLYVTVQHKKLRTPLNYILLNLAVA**N**LFMVFGGFTTTLYTSLHA
Balaenoptera physalus	GFPINFLTLYVTVQHKKLRTPLNYILLNLAVA**N**LFMVLGGFTTTLYTSLHA
Eschrichtius robustus	GFPINFLTLYVTVQHKKLRTPLNYILLNLAVA**N**LFMVFGGFTTTLYTSLHA
Balaenoptera edeni	SFPINFLTLYVTVQHKKLRTPLNYILLNLAVA**N**LFMVFGGFTTTLYTSLHA
Caperea marginata	GFPINFLTLYVTVQHKKLRTPLNYILLNLAVA**N**LFMVFGGFTTTLYTSLHA
Megaptera novaeangliae	GFPINFLTLYVTVQHKKLRTPLNYILLNLAVA**D**LFMVFGGFTTTLYTSLHA
Balaenoptera acutorostrata	GFPINFLTLYVTVQHKKLRTPLNYILLNLAVA**N**LFMVFGGFTTTLYTSLHA
Eubalaena australis	GFPINFLTLYVTVQHKKLRTPLNYILLNLAVA**N**LFMVFGGFTTTLYTSLHA
Balaenoptera musculus	GFPINFLTLYVTVQHKKLRTPLNYILLNLAVA**N**LFMVFGGFTTTLYTSLHA

Exon 4

	292 **299**
Eubalaena glacialis	VTRMVIIMVVAFLICWLPYASVAFYIFIHQGSDFGPIFMTIP**A**FFAKSS**S**I
Tursiops truncatus	VTRMVIIMVVAFLICWVPYASVAFYIFTHQGSDFGPIFMTIP**S**FFAKSS**S**I
Mesoplodon bidens	VTRMVVIMVVAFLICWVPYASVAFYIFTHQGSNFGPIFMTIP**S**FFAKSS**A**I
Bos taurus	VTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIP**A**FFAKTS**A**V
Physeter macrocephalus	VTRMVIIMVVAFLICWVPYASVAFYIFTHQGSNFGPIFMTVP**S**FFAKSS**A**I
Balaenoptera borealis	VTRMVIIMVVAFLICWVPYASMAFYIFTHQGSNFGPIFMTIP**S**XFAKSS**S**I
Balaena mysticetus	VTRMVVIMVVAFLICWLPYASVAFYIFIHQGSDFGPIFMTIP**A**FFAKSS**S**I
Balaenoptera physalus	VTRMVIIMVVAFLICWVPYASVAFYIFTHQGSNFGPIFMTIP**S**FFAKSS**S**I

Eschrichtius robustus	VTRMVIIMVVAFLICWVPYASVAFYIFTHQGSNFGPIFMTIP**A**FFAKSS**S**I
Balaenoptera edeni	VTRMVIIMVVAFLICWVPYASMAFYIFTHQGSNFGPIFMTIP**S**FFAKSS**S**I
Caperea marginata	VTRMVIIMVVAFLICWVPYASVAFYIFTHQGSNFGPIFMTIP**S**FFAKSS**A**I
Megaptera novaeangliae	VTRMVIIMVVAFLICWVPYASVAFYIFTHQGSNFGPIFMTIP**S**FFAKSS**S**I
Balaenoptera acutorostrata	VTRMVIIMVVAFLICWVPYASVAFYIFTHQGSNFGPIFMTIP**S**XFAKSS**S**I
Eubalaena australis	VTRMVIIMVVAFLICWLPYASVAFYIFIHQGSDFGPIFMTIP**A**XFAKSX**S**I
Balaenoptera musculus	VTRMVIIMVVAFLICWVPYASVAFYIFTHQGSNFGPIFMTIP**S**FFAKSS**S**I

Figure 4-4. Alignment of amino acid sequences deduced from cetacean rod opsin exons 1 and 4 and MWS cone opsin exons 3 and 5. Amino acid substitutions associated with significant wavelength modulation are in bold and numbered. Underlined regions indicate transmembrane (TM) helices.

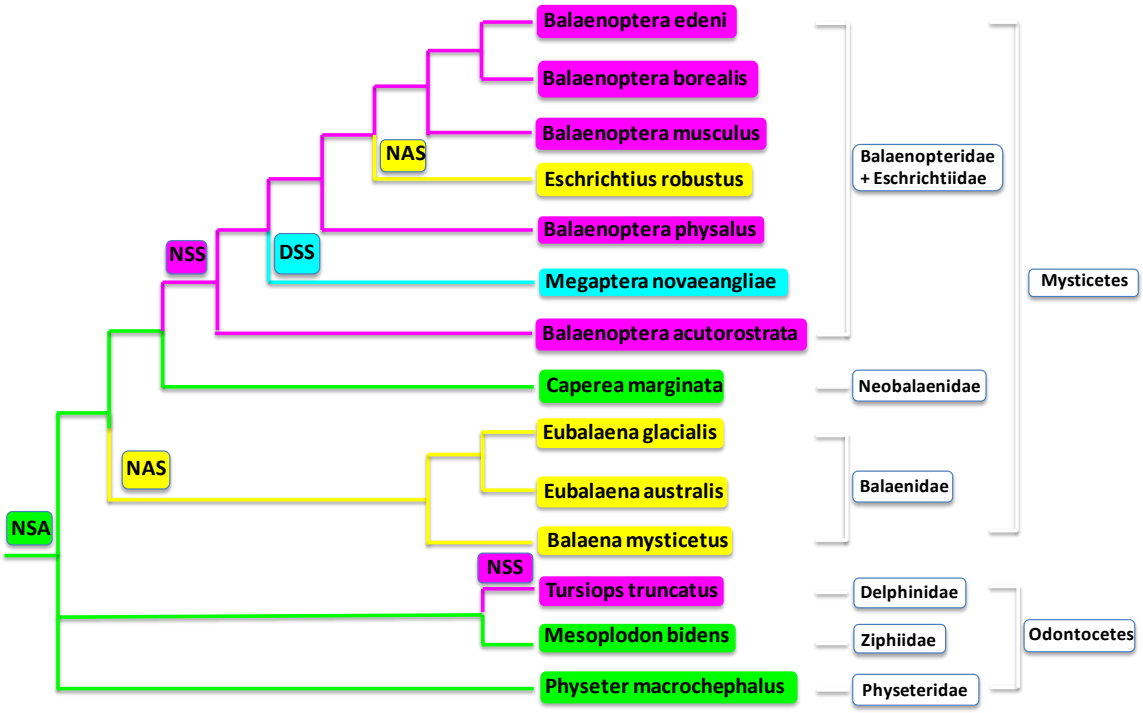

Figure 4-5. Phylogeny of the mysticete whales and the emergence of the amino acid substitutions at positions 83, 292 and 299. Associated absorbance maxima are as follows: NSA, 479 nm, NSS, 484 nm, NAS, 493 nm, DSS, 492 nm. (Note: Terrestrial rhodopsins commonly possess the amino acid substitutions DAA with an absorbance maximum of 500 nm).

Selected References

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Appendix:

Fasick, JI, N Bischoff, S Brennan, S Velasquez, and G Andrade. 2011. Estimated absorbance spectra of the visual pigments of the North Atlantic right whale (*Eubalaena glacialis*). Marine Mammal Science 27(4): E321-331.